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 101  CAACCCGTTG AAAAGCAAAT AGCGGGAGAT ATAATACGTG TACTAGAATT
 151  CAAGCAAACA AATGAAAGTC ATACAGGATT GTATGGAATT GCATATCGAG
                                        TOX F2
 201  CTAAGAAAGT AATAATAGCA TATGCTTTAG CGGTAAGTGG TATTCATAAT
 251  GTCTCTCAAC TTCCAGAAGA CTATTATAAA AATAAGGATA ACACAGGTAG
 301  AATTTATCAA GAATACATGT CTAATCTTTT ATCTGCACTA TTGGGTGAGA
 351  ATGGTGATCA AATTTCTAAA GATATGGCAA ATGATTTTAC CCAGAACGAA
 401  CTGGAGTTTG GAGGTCAACG TCTTAAAAAT ACCTGGGATA TTCCTGATCT
 451  TGAGAATAAA CTATTGGAAG ATTATTCAGA TGAAGATAAA TTATTAGCAC
                                        TOX F1
 501  TATATTTCTT TGCTTCACAA GAACTTCCAA TGGAGGCAAA TCAACAATCA
                                                TOX R3
 551  AATGCAGCAA ATTTTTTTAA AGTAATTGAT TTTTTACTTA TCTTATCTGC
 601  TGTAACATCA CTGGGAAAAA GGATTTTTTC AAAAAATTTT TACAATGGTC
 651  TAGAAACTAA ATCATTAGAG AATTATATTG AGAGAAAAAA ACTTTCTAAA
                                        TOX F3
 701  CCTTTCTTTC GACCACCGCA GAAGTTACCT GATGGCAGAA CAGGCTACTT
 751  GGCCGGTCCA ACAAAAGCGC CTAAATTGCC AACAACGTCT TCTACAGCAA
                                                TOX R4
 801  CAACGTCTAC AGCAGCTTCA TCTAATTGGA GAGTTAGTTT GCAAAAACTT
 851  AGAGATAACC CATCCAGAAA TACATTTATG AAAATGGATG ATGCTGCAAA
 901  ACGAAAATAT AGTTCATTTA TAAAAGAGGT ACAAAAGGGT AATGATCCAC
 951  GTGCAGCAGC AGCAAGTATT GGTACAAAAA GCGGCAGTAA CTTCGAAAAA
1001  CTGCAAGGTA GAGATTTATA TAGTATAAGA CTAAGCCAAG AACACAGGGT
                                        A24AC1
1051  AACATTCTCC ATAAATAATA CTGACCAAAT AATGGAGATC CAAAGTGTTG
1101  GAACTCATTA CCAAAATATA TAACCTGATT TATAGTAGTG ATAAGACGTA
1151  AGATAAATAT GGAAGGTTGT AATTCTATTG CACTTCCTCA GAGGTGACCG
1201  CTCAG
```

FIGURE 1 .

```
  1  MVIKPVTTPS VIQLTPDDRV TPDDKGEYQP VEKQIAGDII RVLEFKQTNE
 51  SHTGLYGIAY RAKKVIIAYA LAVSGIHNVS QLPEDYYKNK DNTGRIYQEY
101  MSNLLSALLG ENGDQISKDM ANDFTQNELE FGGQRLKNTW DIPDLENKLL
151  EDYSDEDKLL ALYFFASQEL PMEANQQSNA ANFFKVIDFL LILSAVTSLG
201  KRIFSKNFYN GLETKSLENY IERKKLSKPF FRPPQKLPDG RTGYLAGPTK
251  APKLPTTSST ATTSTAASSN WRVSLQKLRD NPSRNTFMKM DDAAKRKYSS
301  FIKEVQKGND PRAAAASIGT KSGSNFEKLQ GRDLYSIRLS QEHRVTFSIN
351  NTDQIMEIQS VGTHYQNI
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FIGURE 2

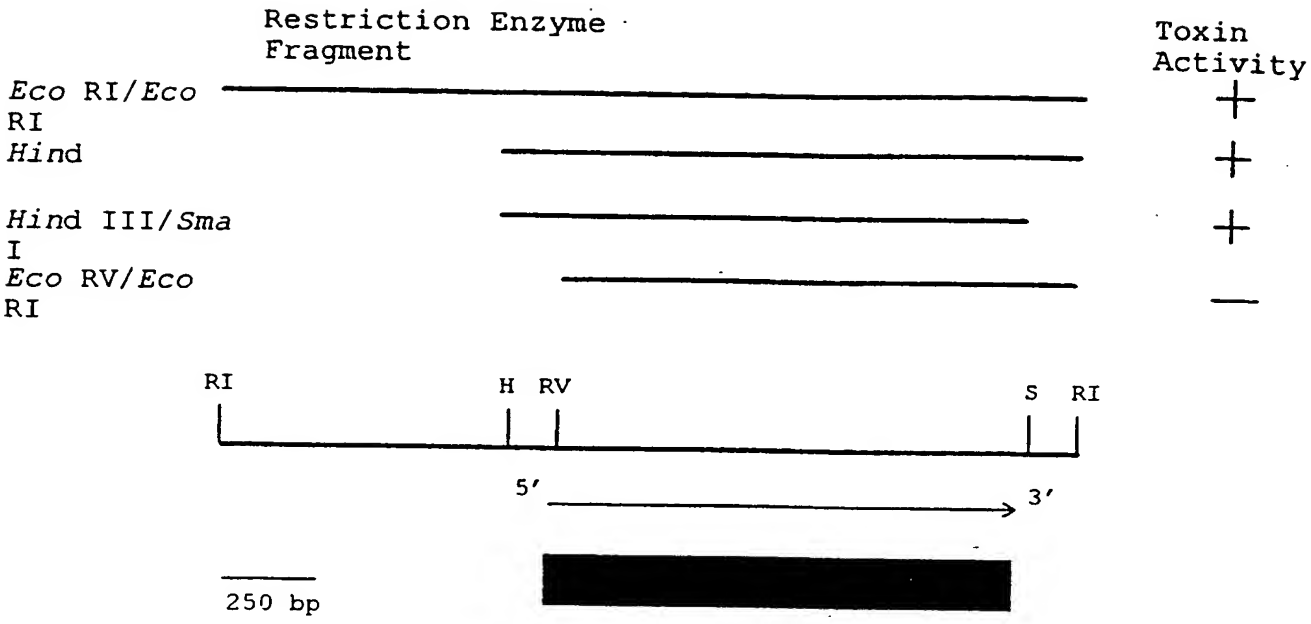
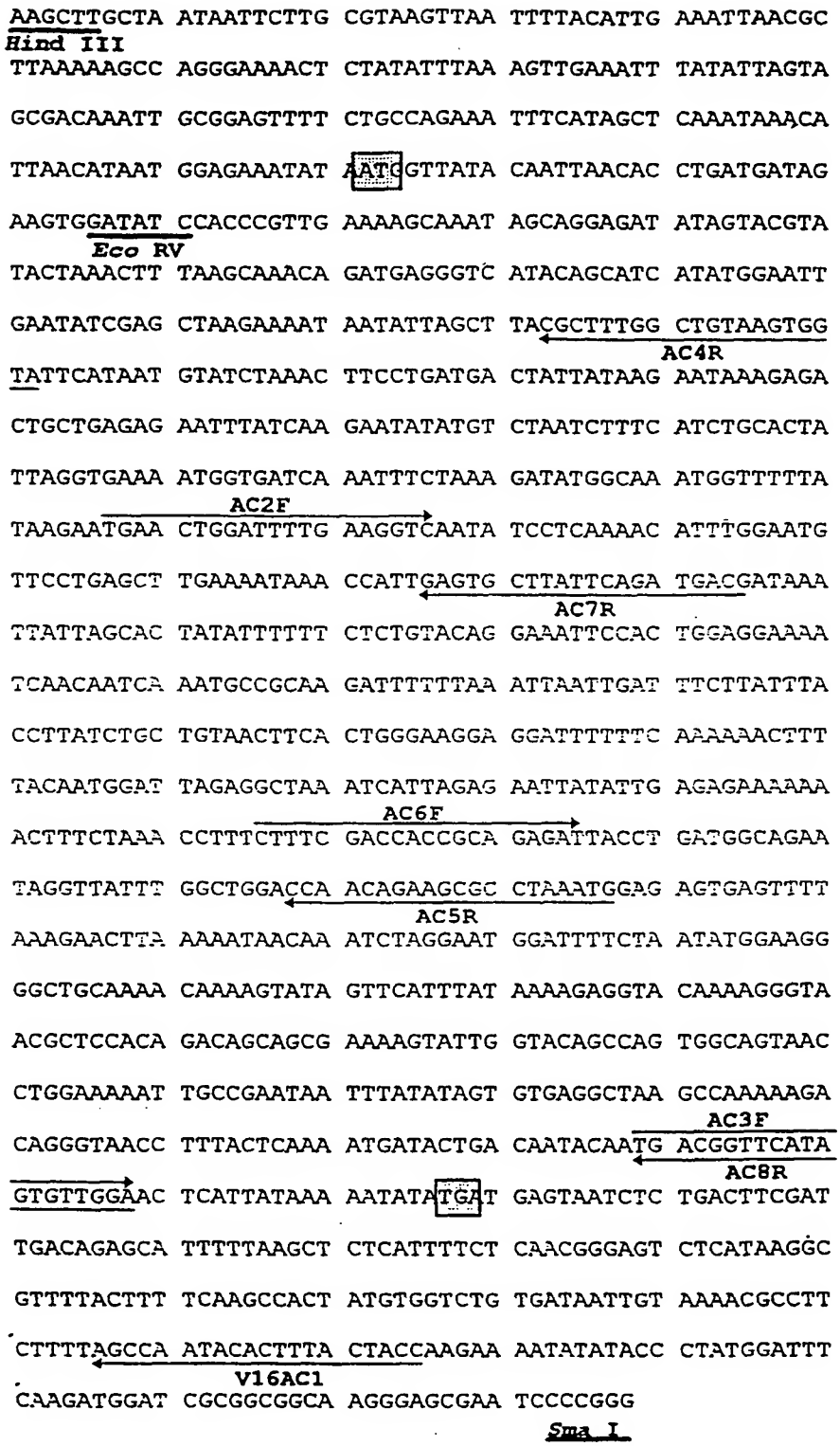

Restriction Enzyme Fragment
Toxin Activity
Eco RI/*Eco* RI
Hind
Hind III/*Sma* I
Eco RV/*Eco* RI
+
+
+
—
RI
H
RV
S
RI
5′
3′
250 bp

FIGURE 3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Hind III
51 TTAAAAAGCC AGGGAAAACT CTATATTTAA AGTTGAAATT TATATTAGTA
101 GCGACAAATT GCGGAGTTTT CTGCCAGAAA TTTCATAGCT CAAATAAACA
151 TTAACATAAT GGAGAAATAT AATGGTTATA CAATTAACAC CTGATGATAG
201 AAGTGGATAT CCACCCGTTG AAAAGCAAAT AGCAGGAGAT ATAGTACGTA
Eco RV
251 TACTAAACTT TAAGCAAACA GATGAGGGTC ATACAGCATC ATATGGAATT
301 GAATATCGAG CTAAGAAAAT AATATTAGCT TACGCTTTGG CTGTAAGTGG
AC4R
351 TATTCATAAT GTATCTAAAC TTCCTGATGA CTATTATAAG AATAAAGAGA
401 CTGCTGAGAG AATTTATCAA GAATATATGT CTAATCTTTC ATCTGCACTA
451 TTAGGTGAAA ATGGTGATCA AATTTCTAAA GATATGGCAA ATGGTTTTTA
AC2F
501 TAAGAATGAA CTGGATTTTG AAGGTCAATA TCCTCAAAAC ATTTGGAATG
551 TTCCTGAGCT TGAAAATAAA CCATTGAGTG CTTATTCAGA TGACGATAAA
AC7R
601 TTATTAGCAC TATATTTTTT CTCTGTACAG GAAATTCCAC TGGAGGAAAA
651 TCAACAATCA AATGCCGCAA GATTTTTTAA ATTAATTGAT TTCTTATTTA
701 CCTTATCTGC TGTAACTTCA CTGGGAAGGA GGATTTTTTC AAAAAACTTT
751 TACAATGGAT TAGAGGCTAA ATCATTAGAG AATTATATTG AGAGAAAAAA
AC6F
801 ACTTTCTAAA CCTTTCTTTC GACCACCGCA GAGATTACCT GATGGCAGAA
851 TAGGTTATTT GGCTGGACCA ACAGAAGCGC CTAAATGGAG AGTGAGTTTT
AC5R
901 AAAGAACTTA AAAATAACAA ATCTAGGAAT GGATTTTCTA ATATGGAAGG
951 GGCTGCAAAA CAAAAGTATA GTTCATTTAT AAAAGAGGTA CAAAAGGGTA
1001 ACGCTCCACA GACAGCAGCG AAAAGTATTG GTACAGCCAG TGGCAGTAAC
1051 CTGGAAAAAT TGCCGAATAA TTTATATAGT GTGAGGCTAA GCCAAAAAGA
AC3F
1101 CAGGGTAACC TTTACTCAAA ATGATACTGA CAATACAATG ACGGTTCATA
AC8R
1151 GTGTTGGAAC TCATTATAAA AATATATGAT GAGTAATCTC TGACTTCGAT
1201 TGACAGAGCA TTTTTAAGCT CTCATTTTCT CAACGGGAGT CTCATAAGGC
1251 GTTTTACTTT TCAAGCCACT ATGTGGTCTG TGATAATTGT AAAACGCCTT
1301 CTTTTAGCCA ATACACTTTA CTACCAAGAA AATATATACC CTATGGATTT
V16AC1
1351 CAAGATGGAT CGCGGCGGCA AGGGAGCGAA TCCCCGGG
Sma I

FIGURE 4

```
  1  MVIQLTPDDR SGYPPVEKQI AGDIVRILNF KQTDEGHTAS YGIEYRAKKI
 51  ILAYALAVSG IHNVSKLPDD YYKNKETAER IYQEYMSNLS SALLGENGDQ
101  ISKDMANGFY KNELDFEGQY PQNIWNVPEL ENKPLSAYSD DDKLLALYFF
151  SVQEIPLEEN QQSNAARFFK LIDFLFTLSA VTSLGRRIFS KNFYNGLEAK
201  SLENYIERKK LSKPFFRPPQ RLPDGRIGYL AGPTEAPKWR VSFKELKNNK
251  SRNGFSNMEG AAKQKYSSFI KEVQKGNAPQ TAAKSIGTAS GSNLEKLPNN
301  LYSVRLSQKD RVTFTQNDTD NTMTVHSVGT HYKNI
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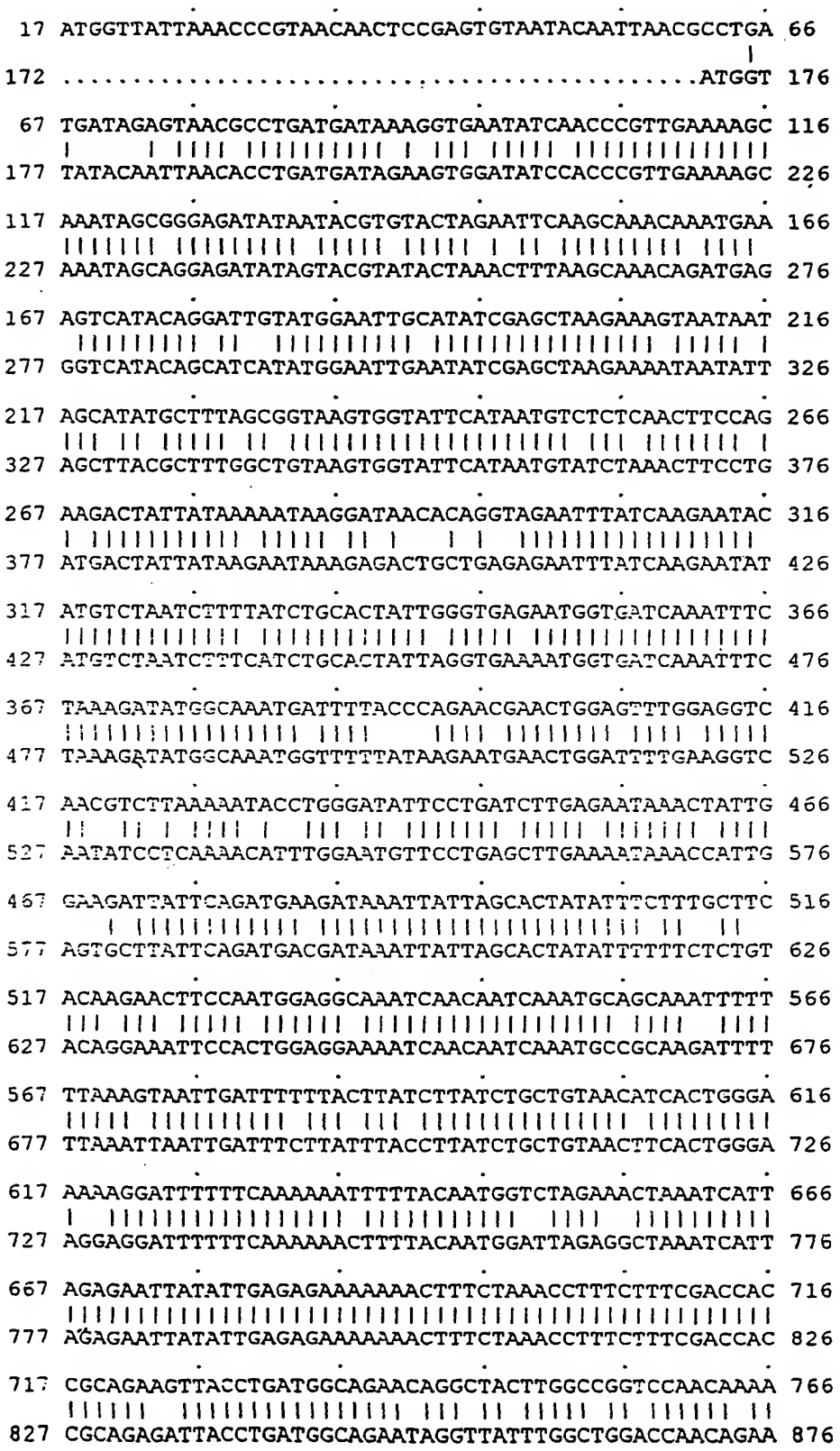
FIGURE 5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172 .....ATGGT 176
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177 TATACAATTAACACCTGATGATAGAAGTGGATATCCACCCGTTGAAAAGC 226
117 AAATAGCGGGAGATATAATACGTGTACTAGAATTCAAGCAAACAAATGAA 166
227 AAATAGCAGGAGATATAGTACGTATACTAAACTTTAAGCAAACAGATGAG 276
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217 AGCATATGCTTTAGCGGTAAGTGGTATTCATAATGTCTCTCAACTTCCAG 266
327 AGCTTACGCTTTGGCTGTAAGTGGTATTCATAATGTATCTAAACTTCCTG 376
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417 AACGTCTTAAAAATACCTGGGATATTCCTGATCTTGAGAATAAACTATTG 466
527 AATATCCTCAAAACATTTGGAATGTTCCTGAGCTTGAAAATAAACCATTG 576
467 GAAGATTATTCAGATGAAGATAAATTATTAGCACTATATTTCTTTGCTTC 516
577 AGTGCTTATTCAGATGACGATAAATTATTAGCACTATATTTTTCTCTGT 626
517 ACAAGAACTTCCAATGGAGGCAAATCAACAATCAAATGCAGCAAATTTTT 566
627 ACAGGAAATTCCACTGGAGGAAAATCAACAATCAAATGCCGCAAGATTTT 676
567 TTAAAGTAATTGATTTTTTACTTATCTTATCTGCTGTAACATCACTGGGA 616
677 TTAAATTAATTGATTTCTTATTTACCTTATCTGCTGTAACTTCACTGGGA 726
617 AAAAGGATTTTTTCAAAAAATTTTTACAATGGTCTAGAAACTAAATCATT 666
727 AGGAGGATTTTTTCAAAAAACTTTTACAATGGATTAGAGGCTAAATCATT 776
667 AGAGAATTATATTGAGAGAAAAAAACTTTCTAAACCTTTCTTTCGACCAC 716
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Figure 6

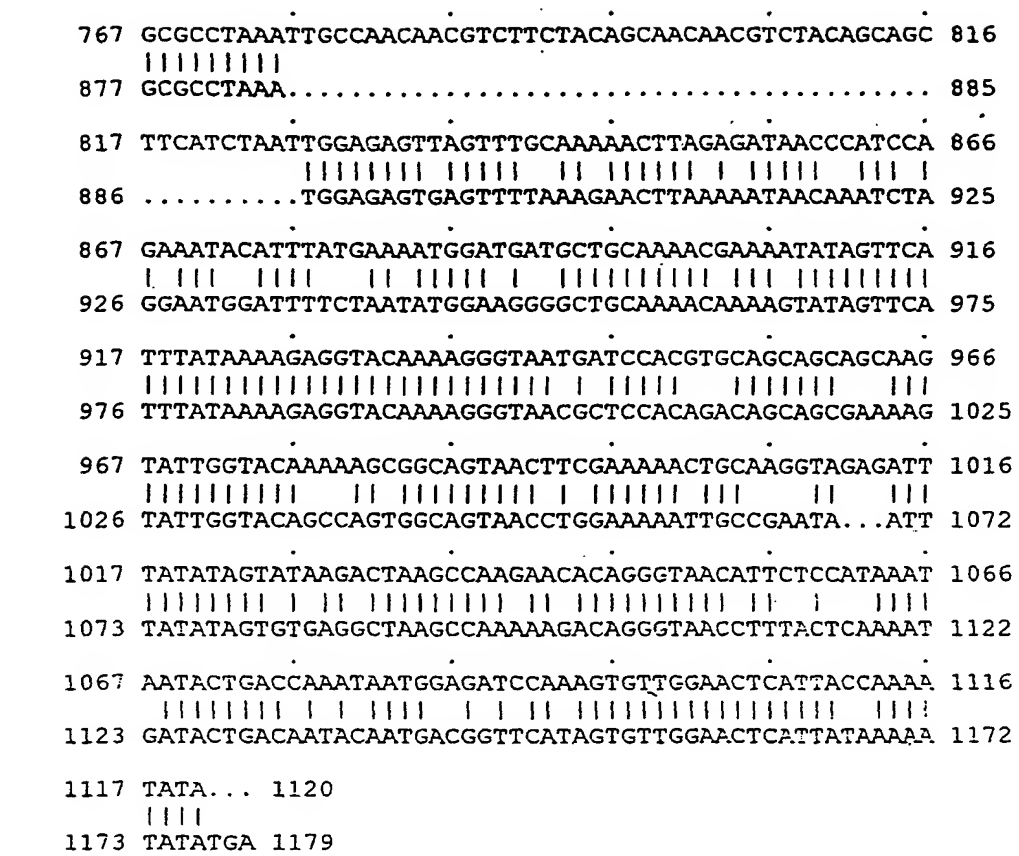
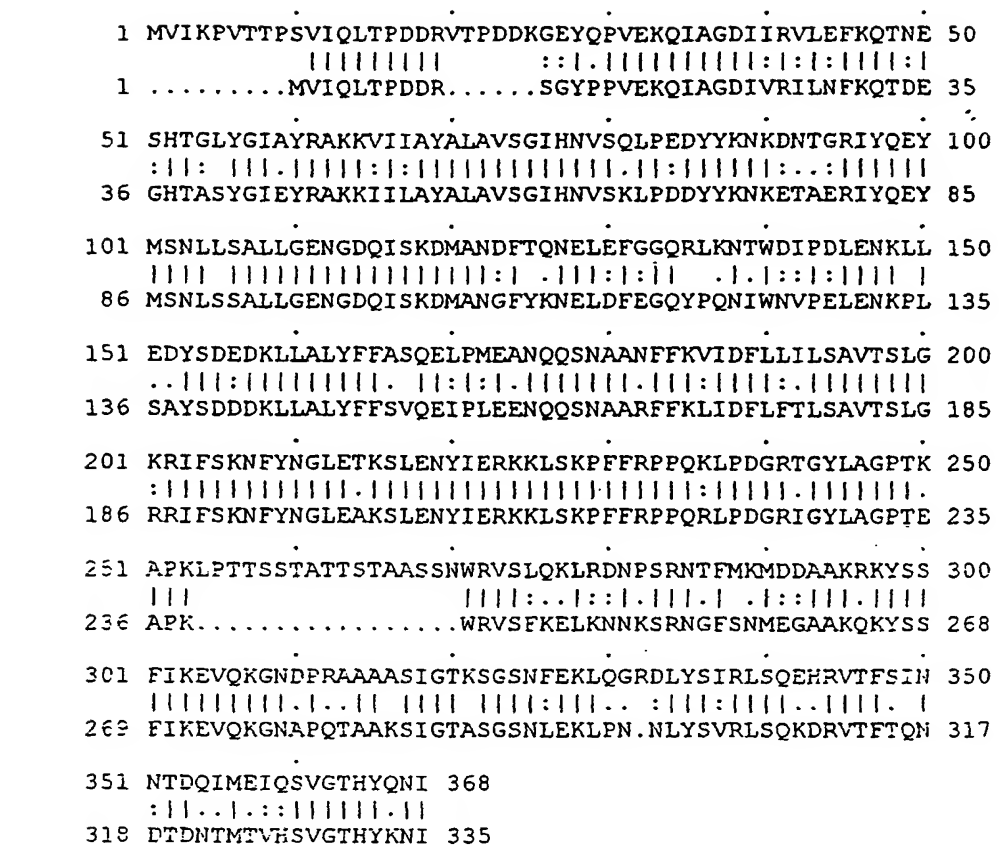


Figure 6 continued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1 .....MVIQLTPDDR.....SGYPPVEKQIAGDIVRILNFKQTDE 35
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36 GHTASYGIEYRAKKIILAYALAVSGIHNVSKLPDDYYKNKETAERIYQEY 85
101 MSNLLSALLGENGDQISKDMANDFTQNELEFGGQRLKNTWDIPDLENKLL 150
86 MSNLSSALLGENGDQISKDMANGFYKNELDFEGQYPQNIWNVPELENKPL 135
151 EDYSDEDKLLALYFFASQELPMEANQQSNAANFFKVIDFLLILSAVTSLG 200
136 SAYSDDDKLLALYFFSVQEIPLEENQQSNAARFFKLIDFLFTLSAVTSLG 185
201 KRIFSKNFYNGLETKSLENYIERKKLSKPFFRPPQKLPDGRTGYLAGPTK 250
186 RRIFSKNFYNGLEAKSLENYIERKKLSKPFFRPPQRLPDGRIGYLAGPTE 235
251 APKLPTTSSTATTSTAASSNWRVSLQKLRDNPSRNTFMKMDDAAKRKYSS 300
236 APK.....WRVSFKELKNNKSRNGFSNMEGAAKQKYSS 268
301 FIKEVQKGNDPRAAAASIGTKSGSNFEKLQGRDLYSIRLSQEHRVTFSIN 350
269 FIKEVQKGNAPQTAAKSIGTASGSNLEKLPN.NLYSVRLSQKDRVTFTQN 317
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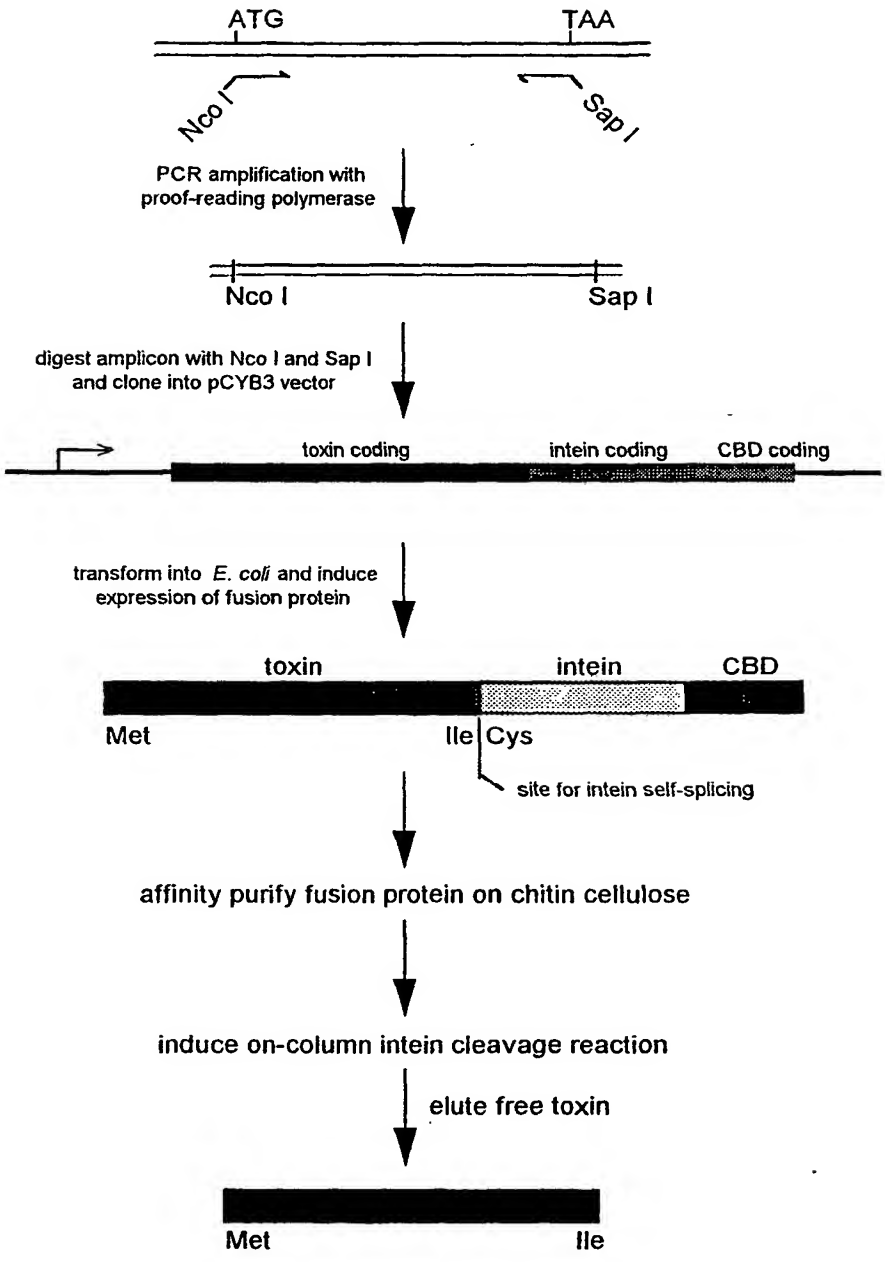
318 DTDNTMTVHSVGTHYKNI 335

FIGURE 7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Sap I
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toxin coding
intein coding
CBD coding
transform into E. coli and induce expression of fusion protein
toxin
intein
CBD
Met
Ile
Cys
site for intein self-splicing
affinity purify fusion protein on chitin cellulose
induce on-column intein cleavage reaction
elute free toxin
Met
Ile

Figure 8.